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Original Research

Investigation of the effectiveness of granulocyte colony-stimulating factor (G-CSF) in the treatment of atrophic rhinitis

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Abstract
Aim: Primary acute rhinitis (AR) remains a clinical problem in developing countries. The objective of this study was to investigate the effectiveness of granulocyte colony-stimulating factor (G-CSF) administration as an alternative to current treatment methods.
Material and Methods: Eighteen rabbits were divided into three groups with six rabbits in each. Group 1 consisted of the rabbits followed up without any treatment as healthy animal group. Pasteurella multocida solution was inoculated into the noses of 12 rabbits in Groups 2 and 3 with a micropipette in order to induce atrophic rhinitis. After three weeks of inoculation, rabbits in Group 1 and Group 2 were sacrificed. Group 3 was administered 5 microgram/kg G-CSF and sacrificed after four weeks. Total turbinate thickness, turbinate bone thickness, turbinate mucosal thickness, degree of cilia loss in epithelial cells, amount of submucosal gland, inflammatory cell infiltration, and changes in the turbinate bones and mucosa were examined.
Results: Total turbinate thickness, turbinate bone thickness and mucosal thickness were statistically significantly lower in Group 2 compared to Group 1 (for all, $p<0.01$). There was a statistically significant increase in the total turbinate thickness, turbinate bone thickness and turbinate mucosa thickness of the rabbits that we treated with G-CSF (Group 3), compared to the rabbits that we created an atrophic rhinitis model and did not give treatment (Group 2) (for all, $p<0.01$).
Discussion: We obtained findings supporting the use of G-CSF as an easy-to-apply treatment method in the medical treatment of atrophic rhinitis.

Keywords
Atrophic Rhinitis, Pasteurella Multocida, Turbinate, Mucosa, Nasal Cavity

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Introduction

Atrophic rhinitis is a chronic disorder characterized by progressive atrophy of nasal and underlying bones of the turbinates with the presence of dried crusts and a foul odor called ozaena. Specific infections, especially those caused by K. ozaenae, Proteus vulgaris, Escherichia coli, Pasteurella Multocida, chronic sinus infection, hormonal imbalance, iron deficiency anemia, malnutrition, autoimmunity and heredity are the blamed factors for the etiology of AR, although the exact cause is unclear [1]. Its incidence in western countries has decreased owing to improved socioeconomic conditions, while it remains an important public health problem in Asia and Africa continents.

AR is divided into primary atrophic rhinitis and secondary atrophic rhinitis. Primary AR is seen in people without any previous nasal disorder and is characterized by sclerotic transformation of the mucous membrane and increased patency of the nasal passage due to atrophic changes [2]. This form of AR accounts for the majority of cases [3]. Secondary AR is less common and may occur after total turbinectomy, septoplasty, endoscopic sinus surgery, and tumor surgery [4]. The diagnosis of AR is clinical and the marked findings on examination include nasal crusts filling the cavity, foul odor and increased volume of the nasal cavity because of the shrinkage of the turbinates [5].

For many years, several medical and surgical methods have been attempted for the treatment of AR. The main goals of the treatment are to reduce secondary bacterial infections and to clean the crusts. Medical treatment of AR includes treatment of injection with antibiotics, regular nasal douching with a solution of dilute sodium chloride, sodium bicarbonate and sodium borate, iron and vitamin supplementation [6]. Surgical treatment methods include complete closure of anterior nares by surgical intervention, also known as Young's operation, partial closure of anterior nares, implantation and denervation operations and turbinate reconstruction [7].

Granulocyte colony-stimulating factor (G-CSF) is a large family of polypeptide molecules that regulate cell division in various tissues by autocrine or paracrine mechanisms. G-CSF plays an important role not only in embryonic development, but also in adult tissue homeostasis, and especially in processes such as wound healing and tumorigenesis [8]. G-CSF is involved in the mobilization of neutrophils from the bone marrow into circulation and is successfully used in the treatment of neutropenia [9].

Understanding the effect of G-CSF on the mobilization of stem cells, which is its natural function, and the idea that mobilized stem cells are directed to damaged tissues and accelerate healing, attracted the attention of many researchers and prompted them to conduct experimental studies on different issues. Studies on G-CSF are continuing in the field of otorhinolaryngology, especially in otology, wound healing, oncology and rhinology [10]. The objective of this study was to investigate the effectiveness of granulocyte colony stimulating factor (G-CSF) administration as an alternative to current treatment methods.

Material and Methods

This experimental study was conducted in the Istanbul University Cerrahpasa Medical Faculty Experimental Animal Production and Research Laboratory after receiving approval from the Istanbul University Cerrahpasa Medical Faculty Experimental Animals Ethics Committee. The study was performed following the Declaration of Helsinki, institutional and national animal rights.

Eighteen white young adult male New Zealand rabbits aged 14-16 weeks and weighing 2.3-3.6 kg were used. The rabbits were fed with the same foods under the same laboratory conditions during the study. The animals were divided into three groups with six rabbits in each. Group 1 consisted of the rabbits followed-up without any treatment as the healthy animal group. Pasteurella multocida solution was inoculated into the noses of 12 rabbits in Groups 2 and 3 with a micropipette in order to induce atrophic rhinitis. After three weeks of the inoculation, rabbits in Group 1 and Group 2 were sacrificed with euthanasia using i.v Xylazin 20 mg/kg. The nasal cavity, nasal septum, paranasal sinus and turbinates in the nasal cavity of the rabbits in these groups were removed as a block and sent for pathological examination in 10% formaldehyde.

After the occurrence of atrophic rhinitis was confirmed in Group 2 by histopathological examination, the rabbits in Group 3 were administered 5 microgram/kg G-CSF (Neupogen®, Filgrastim [G-CSF] Recombinant - methionyl human granulocyte colony-stimulating factor [r-metHuG-CSF]) subcutaneously with an insulin injector to the neck. After the end of four weeks, the rabbits were sacrificed with the same method, the nasal cavity, nasal septum, paranasal sinus and turbinates in the nasal cavity were removed as a block and sent for pathological examination in 10% formaldehyde.

Induction of atrophic rhinitis with Pasteurella Multocida

Passages of Pasteurella Multocida were taken to the bloody nutrient broths. Colonies in the bloody plate medium, which grew in an oven at 37oC for 24 hours, were taken into physiological saline and homogenized by vortexing. It was adjusted to 1010/1 mL of bacteria in the homogeneous suspension by measuring with the DENSIMAT device (Bio Merieux sa France).

Histopathological Examination

The samples were fixed in buffered 10% formalin overnight and were then decalcified in a strong acid solution. Five micron-thick sections were taken from the tissues embedded in the paraffin blocks and stained with Hematoxylin & Eosin (HE) and periodic acid schiff (PAS). The sections were evaluated under a light microscope by the same pathologist. In sections of each rabbit, total turbinate thickness, turbinate bone thickness, turbinate mucosal thickness, degree of cilia loss in the epithelial cells, amount of submucosal gland, inflammatory cell infiltration, and changes in the turbinate bones and mucosa were examined.

In the histopathological examination, each parameter was divided into classes among themselves. Scoring was done semi-quantitatively for each parameter using a score of "0" if there was no congestion, "1" if there was, and "0" if there was no submucosal gland, and "1" if there was.

Statistical Analysis

Statistical analysis of the data obtained was carried out using SPSS version 25.0 (SPSS, Statistical Package for Social Sciences, IBM Inc., Armonk, NY, USA) software. As the variables were skewed, the Mann-Whitney U test was used for

the comparison of continuous variables between the groups. Qualitative data were compared with Fisher's Exact Chi-square test. Continuous variables were expressed as mean ± standard deviation and median values, while categorical variables were given as numbers and percentages. The results were evaluated at a 95% confidence interval and $p<0.05$ statistical significance level.

Results

All of the rabbits (Group 2) infected with Pasteurella multocida developed atrophy in both the bone structure and mucosa of their turbinates compared to the healthy control group (Group 1). Total turbinate thickness, turbinate bone thickness and mucosal thickness were statistically significantly lower in Group 2 compared to Group 1 (for all, $p<0.01$). In addition, loss of submucosal glands, non-specific inflammatory cells in the lamina propria and epithelium, loss of bone trabeculae, and irregularities on the bone surface were found in Group 2 (Table 1). Figure 1 shows the turbinate bone and mucosa of the control group (Group 1), while Figure 2 shows the turbinate bone and mucosa of Group 2, which was infected by Pasteurella multocida.

It was seen that there was a statistically significant increase in the total turbinate thickness, turbinate bone thickness and turbinate mucosa thickness of the rabbits that we treated with G-CSF, compared to the rabbits in which we created an atrophic rhinitis model and did not give treatment (Table 2). In the histopathological examinations of the treatment group, a significant increase in the number of submucosal glands was also observed. Both the thickness of the turbinate bones

Table 1. Comparison of the turbinates and submucosal glands between Group 1 and Group 2

	Group 1		Group 2		p
	Mean±SD	Median	Mean±SD	Median	
Total Turbinate Thickness	46.33±3.83	46.50	27.50±3.33	34.50	0.004**
Turbinate Bone Thickness	13.00±1.79	13.50	9.33±0.82	9.50	0.007**
Mucosa Thickness	33.33±3.88	35.00	18.17±3.25	19.00	0.004**
	n	%	n	%	
Mucous Gland	6	100.0	-	-	0.001**
Congestion	6	100.0	6	100.0	-

** p<0.01 Mann-Whitney U test

Table 2. Comparison of the turbinates and submucosal glands between Group 2 and Group 3

	Group 2		Group 3		p
	Mean±SD	Median	Mean±SD	Median	
Total Turbinate Thickness	27.50±3.33	34.50	33.83±2.14	34.50	0.007**
Turbinate Bone Thickness	9.33±0.82	9.50	12.00±1.67	12.50	0.012**
Mucosa Thickness	18.17±3.25	19.00	21.83±1.94	21.00	0.041**
	n	%	n	%	
Mucous Gland	-	-	6	100.0	0.001**
Congestion	6	100.0	6	100.0	-

** p<0.01 Mann-Whitney U test

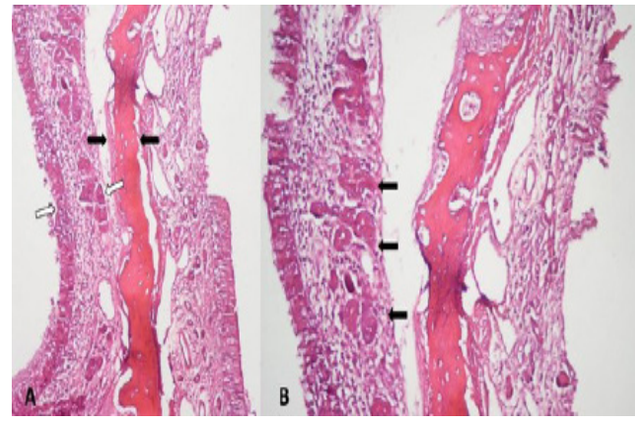

Figure 1. Conchal histological section of the control group (Group 1) rabbits, which were followed without any treatment. A. Normal bone thickness (black arrows) and mucosa with cilia (white arrows) are observed (H&E x 40). B. Submucosal glands (black arrows) (H&E x 200).

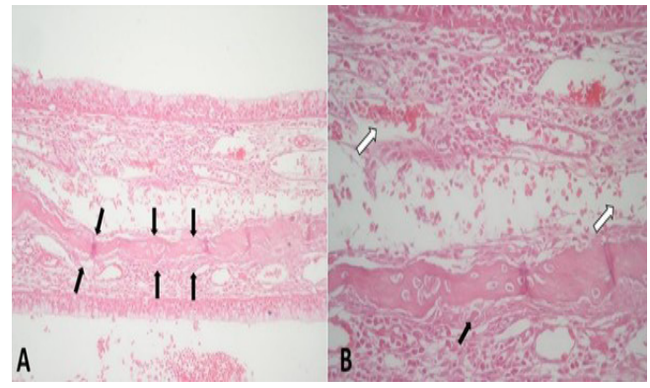

Figure 2. Conchal histological section of Group 2 rabbits with atrophic rhinitis induced by infection of Pasteurella multocida. A. Arrows indicate bone thickness. There is a severe reduction in bone trabeculae compared to normal rabbits. Turbinate bones appear irregular (H&E x 200). B. Nonspecific inflammatory cells in the lamina propria and epithelium (white arrows) and reduced bone trabeculae and periosteal fibroplasia (black arrow) (H&E x 400).

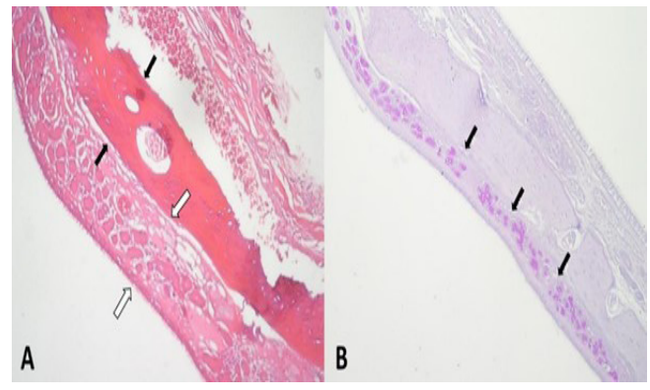

Figure 3. Conchal histological section of Group 3 rabbits with atrophic rhinitis induced by infection of Pasteurella multocida and treated with G-CSF. A. Arrows indicate bone thickness. There is a thickening of bone trabeculae compared to Group 2 rabbits. Turbinate bones appear regular (H&E x 200). B. Arrows show the submucosal glands that occurred again (PAS x 100).

increased and the irregularities on the bone surface decreased (Figure 3).

Discussion

Atrophic rhinitis (AR) is a well-known disease, which dates back to the medical papyri of the Egyptian era. AR was described for the first time by Fraenkel in 1876 as a distinct clinical entity of the nose. Histopathological studies show that AR is a progressive inflammatory process with atrophy and fibrosis, and atrophic changes occur in all parts of the nose [2]. In the present study, we observed histopathological atrophic changes in the Group 2, in which we induced rhinitis with the inoculation of Pasteurella multocida, which is among the causative agents of primary AR [11]. We found that in all rabbits infected with Pasteurella Multocida, the turbinates were significantly thinned in total, mucosal and bone thickness, nonspecific inflammatory cell infiltration occurred in the mucosa and submucosa, and the submucosal glands were atrophied.

Primary AR is less common in developed countries, while it remains a clinical problem in developing and underdeveloped countries [12]. Whereas, secondary atrophic rhinitis tends to increase in developed countries due to the increase in intranasal surgeries and related complications [13]. There are several treatment methods in both forms of AR, with the first-line treatment being conservative.

The goal of conservative treatment is to reduce secondary bacterial infections and clear up crusts. A variety of medical treatments have been suggested for the treatment of primary AR, including nasal wash with anhydrous glucose in glycerin or chloromycetin in the nasal drop and systemic administration of streptomycin, vitamins, minerals, hormones and placental extract [14]. Mechanical crust removal and nasal irrigation under the guidance of endoscopy are an important part of the treatment.

Surgical procedures are performed in severe cases or when medical treatment fails [15]. Numerous surgical treatment methods have been attempted, including reduction of nasal cavity size, increasing the regeneration of the nasal mucosa, increasing the moistening of the nasal mucosa, and increasing the vascularity of the nasal cavity [13]. However, despite all these surgical methods, the results have not been completely satisfactory, some techniques have failed or the disease has recurred. In addition, almost all of these procedures change the normal nasal anatomy even more.

Patients with AR must maintain nasal hygiene regularly with various solutions. Many solutions have been recommended to maintain nasal hygiene. No superiority of one solution over the other has been reported [16, 17]. The most common form of systemic treatment is antibiotics. Rifampicin and quinolones have the broadest spectrum of action covering the wide spectrum of microorganisms extracted from patients with AR [18]. Placental extract for the treatment of AR was first described in 1971 by Sinha et al., initiating increased host deficiency, vascularization, vasodilatation and cellular immunomodulation [19]. However, the first improvement after starting treatment either worsened at the end of treatment or did not continue after the end of treatment in all patients who received placental extract therapy [14]. The first approach is to treat its symptoms in the treatment of AR [20]. But this approach is often disappointing because most patients are dissatisfied with medical treatment. Medical treatment of AR is limited to palliation, it can relieve but is far from curative.

In this study, for the first time in the literature, we investigated the effectiveness of G-CSF therapy, which has been experimentally studied in many different areas, in the medical treatment of AR, the cause of which is still unknown and the treatment of which is the subject of research. Hematopoietic cytokines such as G-CSF and erythropoietin mobilize bone marrow stem cells, enabling differentiation and development of hematopoietic progenitor cells [21]. Stimulation of G-CSF increases granulocytic phagocytosis, chemotaxis, and microbicide activities in infection in response to inflammatory disorders [22]. Bone marrow stromal cells, monocytes, endothelial cells, mesothelial cells, and platelets can produce G-CSF. Understanding the effect of G-CSF on the mobilization of stem cells, which is its natural function, and the idea that mobilized stem cells are directed to damaged tissues and accelerate healing, attracted the attention of many researchers and prompted them to conduct experimental studies on different issues. In an experimental study, Schabitz et al. showed that G-CSF reduced the infarct area by 47% when given within the first 30 minutes in rats with stroke due to middle cerebral artery occlusion [23]. Kawada et al. demonstrated that G-CSF increased the cytokines and provided the regeneration of neuronal cells in rats [24]. G-CSF has been successfully used in the treatment of neutropenia. Furthermore, G-CSF has been studied in numerous medical disorders, including oncology, encephalopathy, heart failure and cerebral ischemia [25].

In the present study, we found that the rabbits in Group 3, in which we created an atrophic rhinitis model and treated with G-CSF, had a significant thickening in both the total turbinate thickness, the turbinate mucosal thickness, and the turbinate bone thickness, compared to the rabbits in Group 2, in which atrophic rhinitis was induced with the inoculation of Pasteurella multocida, but no treatment was administered. In addition, histopathological examinations revealed that the submucous glands lost in the subjects with AR in Group 3 regenerated, the smooth structure of the turbinate bone surface was re-formed, and the ciliary structure was closer to normal.

Conclusion

An ideal method to physiologically correct the impaired intranasal anatomy due to various reasons should be a simple and easily applicable treatment instead of surgical treatments that can make this situation worse. In our experimental study, we obtained findings supporting the use of G-CSF as an easy to apply treatment method in the medical treatment of atrophic rhinitis. However, further studies are needed to use this treatment method in routine ENT practice. We think that our study will become a guide for future studies on this issue.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with

the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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